# INFLUENCE OF SPECIFIC FOOD GROUPS AND WEIGHT ON BREAST CANCER RISK IN POSTMENOPAUSAL AFRICAN AMERICAN WOMEN

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We examined the influence of specific food groups and weight on breast cancer risk in a group of postmenopausal women. In a case control design, 103 African American women (34 cases and 69 controls) were recruited to participate in this study. Mean age was 67 years and mean educational attainment was 12.8 years. From a validated food frequency questionnaire, seven food groups were created and examined: dairy products; whole grain breads and cereals; fats and oils; high fat processed meats; red meats; fruits; and vegetables. Body weight was measured as body mass index (BMI). A logistic regression model was used to adjust for potential confounding variables (i.e., age, education, income, total energy, total alcohol intake) and to determine those factors that influence breast cancer risk. A protective effect from breast cancer was not seen for consumption of fruits and vegetables. Our study revealed that breast cancer risk was positively influenced by consumption of high fat processed meats p=.0003. Women in the upper quartile of processed meat consumption, as compared with the lowest quartile, had an odds ratio of 10.356 (95% CI: 2.078,51.616). This food group consisted of processed foods high in fat such as bacon, breakfast sausage, scrapple, regular luncheon meats, hot dogs and sausage. Women with a BMI >30 (obesity), as compared with a BMI < 25 (normal weight), had an odds ratio of 5.996 (95% CI: 1.012,32.212). Studies have shown conflicting results regarding meat consumption and breast cancer risk. However, we found a significant relationship between processed meat consumption and breast cancer risk. Since processed meats are typically fried in our population (i.e., scrapple, sausage), increased risk may be related to increased exposure to heterocyclic aromatic amines formed from cooking meats at high temperatures. Another explanation could be the high content of saturated fats, additives and preservatives in these foods. Our findings suggest that a high consumption of processed meats and obesity, may partially explain breast cancer risk in postmenopausal African American women.

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# ESTROGEN BIOSYNTHESIS AND METABOLISM GENE VARIANTS AND BREAST CANCER: FAMILY-BASED GENETIC ASSOCIATION STUDY

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Several previous case control association studies have examined associations between polymorphisms in genes that control endogenous estrogen biosynthesis and metabolism and breast cancer and reported inconsistent findings. Here, we report findings from the first family based association study examining the association between polymorphisms in four key estrogen biosynthesis and metabolism genes (CYP17, CYP19, CYP1B1 and COMT) and female breast cancer. We conducted the study among 278 breast cancer nuclear families (with a total of 679 family members) participating in the Metro New York Registry (MNYR) _ one of the six centers of NCI's Cooperative Family Registry for Breast Cancer Studies. We used recently developed likelihood based statistical methods, which are appropriate for nuclear families with arbitrary structure like ours to examine the allelic associations. We found the CYP19 variant allele with 11 TTTA repeats to be associated with breast cancer in these families. We also found that maternal carrier status of the CYP19 allele(s) with 10 or more TTTA repeats was associated with breast cancer risk in daughters (independent of the daughters' own genotype). We did not find any association of a woman's breast cancer with either her own or her mother's CYP17, CYP1B1 or COMT allelic status. In conclusion, findings from this family based study indicate that a woman's own and/or her maternal carrier status of CYP19 TTTA variant alleles may be related to an elevated risk of breast cancer. These findings, if confirmed in future larger studies, will have important implications.

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# BODY FAT PHENOTYPES, SEX HORMONES, AND BREAST CANCER RISK IN POSTMENOPAUSAL AFRICAN AMERICAN WOMEN

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African-American (AA) women have the highest breast cancer mortality rate in the U.S. Despite reports suggesting that breast cancer in AA women might be a biologically more aggressive disease, AA women, especially postmenopausal AA women, remain one of the least studied populations in this country, with very little known about their sex hormone profile. Recent findings have suggested that body fat distribution may be a better marker for breast cancer risk than degree of obesity. This is a 5-year cross-sectional study to determine the association between body fat phenotypes and sex hormone profile associated with increased breast cancer risk in postmenopausal AA women. Blood samples are collected on two consecutive days for hormone determinations. Other data are also collected to allow us to control for established and possible confounding factors.

The number of postmenopausal AA women interested in participating in this study is currently 560. Of these, 49 are eligible. Only 27 eligible women have completed the study protocol; 3 have LBF (WHR=<0.75), 10 NBF (0.75<WHR=<0.80), and 14 UBF (WHR>0.80) phenotypes. Sixteen are Non-Obese (BMI=<27) and 11 are Obese (BMI>27). Because of our small numbers at this time, all statistical analyses were conducted using student's t-test for unpaired variables. NBF compared to UBF phenotype women, and, Non-Obese compared to Obese women, were not found to be significantly different with respect to age, age at menarche, age at menopause, age at first birth, number of children, and height. Obese women were significantly heavier than Non-Obese women ($p<0.001$); no difference in weight were found between NBF and UBF phenotype women. Both UBF compared to NBF phenotype, and Obese compared to Non-Obese women, had significantly higher BMI ($p<0.001$ and $p<0.05$, respectively). WHR was only significantly higher in UBF compared to NBF phenotype women ($p<0.001$), but not in Obese compared to Non-Obese women. Hormone values for only 20 of these women have already been determined. No significant differences in the levels of estrogens, androgens, sex hormone-binding globulin (SHBG), luteinizing hormone (LH) and follicular stimulating hormone (FSH) between NBF (N=6) and UBF (N=11) phenotype women were found due to the small numbers. However, the same is not true for Non-Obese and Obese women. Even at such low numbers of 11 and 9, respectively, significant differences are observed in certain hormone levels. Obese women had significantly higher levels of estrone (E1; $p=0.008$, by 38%), free estradiol (Free E; $p=0.007$, by 46%), and lower level of FSH ($p=0.047$ by 65%) compared to Non-Obese women. In addition, Obese women also showed close to significantly higher levels of estradiol (E2; $p=0.06$ by 33%), androstenedione (A4; $p=0.056$, by 34%), and lower levels of SHBG ($p=0.069$, by 59%).

Our limited data do suggest that postmenopausal AA women who are obese tend to have a distinct estrogenic/androgenic hormonal profile associated with increased risk of breast cancer. Recruiting postmenopausal AA women for this study continues to be very challenging to our research team. Given more time, we believe we will be able to recruit more women to enable us to determine the relationship between body fat phenotypes and sex hormones associated with increased breast cancer risk in this understudied population. Our study findings can be of great public health significance to help better identify women at high risk for developing breast cancer as well as to more precisely target women for preventative purposes.

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# POLYMORPHISMS/MISSENSE MUTATIONS IN THE ATAXIA TELANGIECTASIA, MUTATED (ATM) GENE IN BREAST CANCER PATIENTS

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Purpose: Mothers of children diagnosed with ataxia telangiectasia have been reported to be at increased risk for breast cancer development. To test whether germline mutations in the ataxia telangiectasia, mutated (ATM) gene are associated with breast cancer, we compared the frequency of ATM cDNA sequence changes in breast cancer patients and controls.

Methods: We sequenced ATM cDNA in 91 breast cancer patients and compared sequence changes in these patients to the frequency of these alterations in a control set of 996 individuals with no cancer history. An allele specific oligonucleotide assay was used to study the specific polymorphisms of interest in the ATM cDNA for the control set. The frequency of identified base changes was also tested across ethnic groups and gender.

Results: No mutations that would lead to protein truncation were identified, but several polymorphisms were found in the cDNA of the breast cancer patients. The three polymorphisms that were found in two or more patients cause amino acid substitutions in the ATM protein of the following type: Ser49Cys, Pro1054Arg, and Asp1853Asn. The Ser49Cys polymorphism was found in 6.7% (5/75) of the breast cancer patients compared to 1.6% (12/946) of the control group (P=0.006, Fisher's 2-sided exact). The subgroup of patients with bilateral breast cancer had a frequency rate of 11.8% (2/17) which again was significantly different from the control group (P=0.025, Fisher's 2-sided exact). None of the 9 breast cancer patients that had a normal tissue complication following radiation treatment had the Ser49Cys change. The allelic frequencies of the other two polymorphisms were not different between cases and controls.

Conclusion: Breast cancer patients, particularly those with bilateral disease, are more likely to have a polymorphism in the ATM gene that results in a Ser49Cys change in the protein compared to controls. These data suggest Ser49Cys may be a functional polymorphism that contributes to breast cancer development or a polymorphism that is linked to another causative genetic factor.

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# INTAKE AND METABOLISM OF B VITAMINS AND RISK OF BREAST CANCER IN THE LONG ISLAND BREAST CANCER STUDY PROJECT

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Breast cancer is thought to be largely preventable through dietary and lifestyle modifications. Micronutrients in diet such as vitamins may modify the risk of breast cancer. Insufficient levels of folate (Vitamin B9) and other B vitamins have been associated with increased risk of breast cancer in several epidemiologic studies. A functional polymorphism in a folate-metabolizing gene, methylenetetrahydrofolate reductase (MTHFR) 677C->T, influences the distribution and bioavailability of these nutrients in the body, thus may modify the risk of breast cancer associated with these nutrients. We are investigating the association of dietary intake of B vitamins in relation to breast cancer risk in the Long Island Breast Cancer Study Project, a large population-based case-control study consisting 1508 cases and 1555 controls. More specifically, we will report our findings on risk of breast cancer associated with dietary intake of folate as well as vitamins B1, B2, B3, B6 and B12. In addition, we will report the association of the MTHFR 677C->T polymorphism and risk of breast cancer and its interactions with dietary B vitamins in relation to breast cancer risk.

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# INSULIN-LIKE GROWTH FACTOR I POLYMORPHISMS AND BREAST CANCER

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Insulin-like growth factor I (IGF-I) is an important regulator of growth and differentiation, is known to inhibit apoptosis, and is one of the most potent mitogens for human breast cancer cells. A number of epidemiologic studies have found that women with higher levels of serum IGF-I show an elevated risk of breast cancer, especially in premenopausal women. While serum IGF-I levels have been studied extensively, very few studies have looked at how IGF-I genotypes may affect breast cancer. This study draws upon an on-going population-based case-control study with specific aims to investigate the role that the IGF-I gene polymorphisms have on breast cancer occurrence.

Studies looking at a polymorphic region of the IGF-I gene containing multiple cytosine-adenine dinucleotides (CA repeats) have shown that different lengths of the CA repeats are associated with varying circulating IGF-I levels. Extracted DNA arising from blood samples collected in 1,087 breast cancer cases and 1,122 control women are being assayed for different lengths of CA repeats. Results from this study will be combined with data collected by the parent study. A separate validation study will be conducted to assess whether the associations for serum IGF-I levels and number of CA repeats found in previous studies is also true in a small sample of our population. In the first step of this study we will determine whether the risk for having CA repeats lengths previously shown to result in higher levels of serum IGF-I is greater in breast cancer cases as compared to controls. Since the parent study provides an assessment of reproductive factors, we will also investigate whether the odds ratio for premenopausal breast cancer in relation to IGF-I gene CA repeats is higher than the odds ratio for postmenopausal breast cancer, as compared to controls. The study is still in the sample preparation phase and has not yet generated data. However, future results gained from this large study may provide evidence indicating a link between IGF-I genotypes and breast cancer risk, and also show that menopausal status confers differential risk of breast cancer in women with particular genotypes.

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# MICROSOMAL EPOXIDE HYDROLASE POLYMORPHISMS AND TOBACCO SMOKING IN RELATION TO RISK OF BREAST CANCER

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Microsomal epoxide hydrolase (mEH) is a detoxifying enzyme involved in the metabolism of environmental and tobacco carcinogens. Genetic polymorphisms within the coding region of the mEPHX gene are known to alter the enzyme's total activity. A polymorphism in exon 3 leads to substitution of tyrosine residue 113 to histidine and is associated with reduced enzyme activity. By contrast, a polymorphism in exon 4 leads to substitution of histidine residue 139 to arginine and is associated with enhanced activity. In this study we examined the association between mEH polymorphism and breast cancer risk in presence of cigarette smoking in pre- and postmenopausal women. In a subset of a case-control study of breast cancer in western New York, 267 women with incident, primary, histologically confirmed breast cancer and 293 community controls were interviewed and a blood specimen obtained. The two polymorphisms of mEH were determined by PCR-RFLP assay. Odds ratios and 95% confidence intervals were calculated using unconditional logistic regression. There was no overall relationship between mEH genotypes and breast cancer risk. Stratification by menopausal status resulted in no significant differences in risk for individuals with polymorphisms at either exon 3 or exon 4, compared to individuals without variant alleles, although there was a slight increase in risk for pre-menopausal women with polymorphism in exon 3 (OR = 1.5; CI= 0.9-2.6). Furthermore, no association was found between breast cancer and mEH genotypes when cases and controls were stratified by both menopausal and smoking status (ever and never smokers). In conclusion, this study suggests that there is no significant association between mEH polymorphisms and breast cancer-risk in general or for groups defined by smoking or menopausal status.

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# THE IMPACT OF RISK FACTORS AND GENETIC POLYMORPHISMS ON BREAST CANCER RISK IN BRCA1 AND BRCA2 MUTATION CARRIERS AND NONCARRIERS—A POPULATION-BASED STUDY

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One mutation in each BRCA gene, a rare BRCA1 mutation and a more frequent BRCA2 mutation, account for about half of familial breast cancer in the Icelandic population. Our previous studies show that the penetrance of the BRCA2 mutation varies greatly, suggesting the involvement of modifying factors, genetic and/or environmental. The aim of the study is to test whether environmental risk factors and genetic variation in metabolic enzymes affect penetrance of BRCA mutations and breast cancer risk in the population in general.

All Icelandic women who have been diagnosed with breast cancer and their close relatives are invited to participate as well as an age-matched comparison group. A total of 2,238 individuals, 868 breast cancer cases, 1,189 relatives and 181 controls, have participated in the study to date, given a blood sample and answered detailed questionnaires. Sample collection is rapid progress and we aim to complete it by October 2002. Screening for mutations in BRCA1 and BRCA2 and polymorphisms in CYP, GST and TP53 genes is in progress. BRCA2 mutation are much more frequent than BRCA1, or 95 compared with 2, in samples screened. Samples from 300 individuals have been screened for the different polymorphisms. Preliminary results indicate that increased number of pregnancies does not give protection against breast cancer in the BRCA2 mutation carriers.

It is important to evaluate the influence of modifying factors on the penetrance of BRCA mutations and the effect of these factors on breast cancer risk in general. There are two main reasons why this population-based study is unique. First, the unusual situation where two founder mutations appear to account for all BRCA related breast cancer. Second, the evidence that a single BRCA2 founder mutation has varied penetrance and expression in carriers in a relatively homogeneous population. This shows that there clearly are modifiers and this population may be ideal for searching for those.

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# DIETARY ANTIOXIDANT VITAMINS C AND E AND BREAST CANCER SURVIVAL

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There are limited clinical and epidemiological studies, which have examined the relationship between antioxidants and risk of breast cancer recurrence or mortality, despite observed protective associations with breast cancer incidence. A meta-analysis of six previous studies revealed that highest intake of vitamin C was modestly and significantly associated with a reduced risk of breast cancer related mortality (Random effects estimate=0.66, 95% CI, 0.48-0.90). We used proportional hazards modeling to estimate rate ratios among 407 postmenopausal women diagnosed with breast cancer between 1986 and 1988 enrolled into a case-control study on diet and cancer. These women were re-contacted with a questionnaire to ascertain use of nutritional supplements during 12 to 14 years of post-diagnosis follow-up time. One or more dietary supplements were used by 80.5% of women, and use of supplements increased significantly after diagnosis. Antioxidant supplement use (vitamin C, E, beta-carotene, selenium or an antioxidant combination) increased from 34% pre-diagnosis to 56% after diagnosis, and was overall used by 64% of this cohort. Vitamin E supplements showed the strongest protective effect on recurrence and mortality when used for more than 3 years (RR=0.40, 95% CI: 0.18-0.90). Exclusion of proxy questionnaires for women who had died during follow-up (N=47) resulted in an expected lessening of effect (RR=0.62, 95% CI: 0.26-1.49). Highest intakes of vitamin E and C from diet alone were not related to risk. Limited modification of risk estimates for vitamin E and recurrence were observed among women who did and did not receive radiation therapy, in which strongest protective effects were found among vitamin E users without radiation therapy. Risks of recurrence and disease related mortality were reduced among women using vitamin E supplements for more than 3 years. Recall bias among proxy respondents may have contributed to these findings. This study provided limited support for the hypothesis that vitamin C and E supplements may reduce the risk of breast cancer recurrence or cancer related mortality.

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# DIFFERENCES IN GENE EXPRESSION OF BREAST CARCINOMAS IN PRE- AND POSTMENOPAUSAL WOMEN

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Background: Evidence indicates that invasive breast carcinomas in pre-menopausal women are more likely to be estrogen receptor negative, of a higher histologic grade, have a higher proliferation rate and confer a worse prognosis than those in post-menopausal women. These findings suggest that there are intrinsic biologic differences in gene expression patterns between breast carcinomas in older women versus younger women. To identify differences in gene expression in invasive breast carcinomas in pre- and post-menopausal women, high density gene expression profiling technology is utilized.

Methods: Snap-frozen tissue from breast carcinomas and from normal tissue in the same breast is collected from 12 pre-menopausal women less than 46 years of age and 12 post-menopausal women older that 64 years of age. For each tissue sample, the diagnosis is confirmed via frozen section histologic analysis. RNA is isolated from multiple cryostat sections of the trimmed tissue using Trizol Reagent (Gibco) followed by RNeasy clean-up (Qiagen). Biotinylated cRNA probes are generated from the isolated RNA and hybridized to high-density oligonucleotide microarrays (Affymetrix U133A Chip interrogating ~16,000 transcripts). Hybridization is detected using a streptavidin-phycoerythrin conjugate and quantified with a high-resolution scanner. Genes that exhibit a statistically significant difference in expression between pre- and post-menopausal women are indentified by conducting an omnibus test of whether the observed distribution of p-values is significantly different from a uniform distribution. The distribution of p-values is modeled as a mixture of beta distributions.

Results: Thus far, breast tissue samples from 3 pre-menopausal women and 6 post-menopausal women have been collected. RNA has been extracted from these samples and they are currently being processed for hybridization. Collection of additional breast tissues continues.

Conclusions: Consistent differences in gene expression in breast carcinomas from pre- and post-menopausal women are likely to impact the applicability of recognized prognostic markers and the relative effectiveness of known and novel therapies in these two groups of patients.

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# ELEVATED MUTATION FREQUENCIES IN CELL LINES FROM BRCA1/2 CARRIERS: A PHENOTYPE INDICATIVE OF DEFICIENT DNA REPAIR AND PREMATURE AGING

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Cancer, including breast cancer, is caused by the accumulation of somatic mutations in oncogenes, tumor suppressor genes and mutator genes. Most cancer-predisposing human diseases involve the loss of fidelity of DNA metabolism or repair. We have shown that these diseases are often characterized by elevated frequencies of in vivo bone marrow somatic mutation. Somatic mutation is an unavoidable consequence of cellular replication and metabolism, and the frequency of in vivo somatic mutation has been shown to significantly increase with age. We obtained immortalized lymphoblastoid cell lines from five carriers of mutations in the breast cancer predisposing BRCA1 gene, four carriers of mutations in the BRCA2 gene and nine normal individuals of widely varying age at time of blood donation. We then applied the drug selective, clonogenic HPRT mutation assay to these cell lines to determine whether carrier status for these potential mutator genes affected their baseline mutation frequencies. First we established that an aging effect indistinguishable from that observed in vivo could be demonstrated amongst the normal cell lines. The slope of the age regression indicates an increase of one new mutation per million cells every four years over lifetime. By first purging the input cell populations of pre-existing mutants we were able to demonstrate that at least two mechanisms contribute to this increase. First, we observed an intrinsic increase in the mutation rate that may be related to an overall loss of cell viability. Second, we found evidence for accumulation of mutants over time. When these same studies were repeated with cell lines established from BRCA1/2 mutation carriers, a much different pattern was evident. Cell lines from young mutation carriers had significantly higher HPRT somatic mutation frequencies than age-matched normal controls, but there was no evidence of any effect of donor age amongst the carrier cell lines. Thus, by age 60 the frequency of somatic mutation in the cell lines from genetically normal individuals were indistinguishable from the mutation carriers. This is the first description of a distinctive cellular phenotype for carriers of BRCA1/2 mutations that could play a role in their susceptibility to breast cancer.

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# PREVALENCE OF POTENTIAL BREAST CANCER SUSCEPTIBILITY GENES (INCLUDING CYP17) IN TWIN PAIRS WITH BREAST CANCER AND CONTROLS

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**Introduction:** A woman with a family history of breast cancer has a higher risk of developing breast cancer than a woman without a family history. Among identical twin pairs, those in which breast cancer has occurred in both twins (concordant pairs) have greater genetic predisposition than other women with a family history. The genetic factors previously identified (i.e., BRCA1/2) do not account for all cases with a genetic risk. Thus, concordant twin pairs are an important group in which other genetic factors can be studied. This study utilizes breast cancer twins from a large registry of twins with cancer to test whether suspected genetic factors occur more frequently in concordant pairs than in pairs with only one member affected (discordant pairs) or in control women (without breast cancer).

**Methods**: Genes related to estrogen and carcinogen metabolism, previously reported as being related to breast cancer risk, have been selected for study. In the estrogen metabolism pathway, polymorphisms have been described in the CYP17 gene, the CYP19 gene, the COMT gene, and the HSD17B1 gene. Genes related to carcinogen metabolism which have been linked to breast cancer risk include GSTM1 and P1 and CYP1A1. Our first results are presented for the CYP17 gene, which has a polymorphism (called A2) which is associated with increased endogenous estrogen levels. DNA has been obtained from tissue blocks that were preserved when the breast cancer was first diagnosed, or from buccal smears from control women. We will ultimately obtain tissue or buccal smears from 200 concordant pairs of identical twins, 200 discordant pairs, and 200 control women. A previous study (DAMD17-94-J-4290) obtained tissue blocks from some of the pairs and additional tissue blocks have been obtained during the current study.

**Results**: To date we have received signed informed consents and tissue blocks or buccal smears from 131 discordant pairs, 94 concordant pairs, and 88 control women. Additional follow-up of twins, completion of consent forms, and recruitment of control women is ongoing. The CYP17 laboratory work has been completed for at least one member of 167 pairs (97 discordant and 70 concordant). Preliminary analysis shows that the proportion of women with at least one A2 allele was higher in the discordant pairs than in the concordant pairs (72% vs. 56%, $p<.05$) and the proportion in concordant pairs was similar to control women from other studies. We have also compared the prevalence of the CYP17 genotype with other tumor characteristics (ER and tumor grade) and breast cancer risk factors (age at diagnosis, family history, age at menarche, parity, OC use) and found no differences.

**Summary:** This suggests that this CYP17 polymorphism is not related to increased risk in the concordant pairs; however final conclusions cannot be made until the study has been completed.

**Conclusions**: These findings, as well as results from the additional genetic factors to be tested, will help determine which other genes may affect breast cancer risk. The DNA from these twins can also be used to test newly identified potential breast cancer susceptibility genes.

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# QUASI-PROSPECTIVE STUDY OF BREAST CANCER, DIET, PHYSICAL ACTIVITY, AND BODY HABITUS

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Epidemiological evidence indicates that conventional risk factors explain 50% of total breast cancer risk and population rates of change in incidence over time. The period of large increases in breast cancer rates has coincided with a period of rapid change in the lives of women, including major changes in patterns of physical activity, preparing and eating food, and increases in the prevalence of overweight. These factors may exert powerful influences on physiologic processes leading to cancer. This quasi-prospective study provides a means for relating physical activity, diet, and adult weight history to the odds of developing breast cancer. A total of about 35,000 women are screened each year using routine mammography in the Breast Care Centers of the Palmetto Richland Memorial and Baptist Hospitals of Palmetto Health /South Carolina Cancer Center (BCC). Over the study recruitment period we project about 1,350 women will have a histologically confirmed breast cancer. Our goal for case recruitment is 648 (48%) of this total. We project that 4 controls will be enrolled per case.

After obtaining permission from the Human Use Review Office of the USAMRAA (on 30 November 2000) to begin recruitment we finished the run-in process and began recruitment in the Baptist Hospital BCC in spring of 2001. Currently, we have recruited approximately 300 patients, primarily at the Baptist site. Recruitment at Richland to begin 4/02) will add another 8 patients/day. The proportion of Blacks at the Richland site is projected to be about 2 to 3 times higher than at Baptist. Thus far, 81% of recruited patients on whom we have complete data are White; 16% are Black. Their average age is 52.5 years; 60% have a high school education or less, and 70% are employed either full or part time. They are primarily a sedentary group, reporting an average of 0.45 hours/week of total vigorous physical activity. Their average body mass index [BMI = weight(kg)/height(m)^2] of 27.2 is consistent with this low level of physical activity, as is an average percent body fat of 36.1%. With recruitment now approaching full capacity we project being able to conduct our first interim analysis of the case-control data by the end of the summer 2002.

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# PHASE I INDUCTION AND ESTROGEN METABOLISM IN WOMEN WITH AND WITHOUT BREAST CANCER AND IN RESPONSE TO A DIETARY INTERVENTION

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In both ecological and clinical studies we have observed potentially protective effects of vegetables in the Brassica genus (e.g., broccoli, cauliflower, brussel sprouts) on risk of breast cancer. Compounds in these foods may modify estrogen metabolism by causing 17b-Estradiol (E2) to be metabolized to 2-hydroxyestrone (2HE) rather than 16a-hydroxyestrone (16HE), the higher-risk metabolite. The indole glucosinolates (IGSL), which are contained in high concentrations in Brassica vegetables, are converted in the body to aryl hydrocarbon receptor (AhR) agonists. The activated AhR induces a number of protein products that can shift E2 metabolism away from 16HE and towards 2HE. AhR activation also induces immune system factors such as interleukin-1b (IL-1b) and other proteins, such as plasminogen activator inhibitor-2 (PAI-2), a protease inhibitor that has been associated with inhibition of tumor invasiveness (metastasis).

In order to examine the relationship between AhR, CYP1B1, IL-1b, PAI-2, estrogen metabolism, and IGSL from Brassica intake in relation to breast cancer risk we will conduct two sequential studies; a cross-sectional comparison and a diet intervention. Our plan is to recruit 90 postmenopausal women, of whom one third will have had breast cancer and to compare these women on: 1) AhR activation and its various protein products relevant to cancer including CYP1B1, PAI-2, and IL-1b; and 2) levels of relevant estrogens, E2, 2HE, and 16HE. Blood and fasting morning urine samples are collected from each woman at recruitment and then following the intervention for the purposes of measuring the estrogens, AhR activation, and levels of PAI-2 and IL-1b. Adipose tissue for assay of CYP1B1 will be collected by fine needle aspiration at the time of recruitment and at follow-up. Currently, we have recruited and obtained baseline samples from 20 women. Of these, 8 have had breast cancer. Their average age is 58.2 years. They are relatively heavy, with an average body mass index [BMI = weight(kg)/height(m)^2] of 28.5; 25% are smokers. The first diet intervention group is beginning in April 2002. Statistical analyses will examine the effect of the indole carbinols by fitting the data as continuous, which takes into account varying levels of compliance.

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# THE RELATIONSHIP BETWEEN WEIGHT HISTORY, MELATONIN, AND STEROID RECEPTOR STATUS IN WOMEN WITH BREAST CANCER

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Obesity and other indicators of adiposity have been shown not only to increase risk of breast cancer, but also to increase the risk of relapse and mortality after breast cancer diagnosis. Given the significance of steroid receptor status on prognosis and the hypothesized link between melatonin and steroid receptor expression, the objective of this investigation was to examine the impact of weight history on these outcomes among women with breast cancer.

172 women aged 20 to 65 years with stage I or II breast cancer were randomized to one of three groups: a 15-session group based, dietitian-led nutrition education program; a mindfulness-based stress reduction clinic program; or standard supportive care. Weight history and pathology were assessed at baseline with serum melatonin levels assessed at baseline and 4 months (immediately post intervention). Logistic regression was used to assess the relationship between weight history and steroid receptor status as well as weight history and serum melatonin levels.

Age at maximum body mass index [BMI=weight(kg) / height (m)^2] appears to modify the relationship between maximum BMI and estrogen receptor (ER) status (p=0.06) such that among women who attain a maximum weight at 40, for each unit increase in BMI, the risk of being estrogen receptor positive decreases by 6%. In comparison, among women who attain a maximum weight at 50, a 1 unit increase in BMI confers an increase in the risk of being estrogen receptor positive by 2%. Progesterone receptor status was effected by maximum weight (p=0.07), amount of weight gain between 18 years and maximum weight (p=0.05), and maximum BMI modified by age at maximum weight (p=0.06). Increased weight decreased the probability of progesterone receptor positivity. Weight gain from 18 years to maximum weight and 18 years to present weight impacted baseline melatonin levels; a 5 pound increase in weight corresponded to a decrease in melatonin levels of 0.63 ug/day (urinary 6-sulphatoxymelatonin; p=0.02) and 0.72 ug/day (p=0.02), respectively. Weight change from baseline to 4 months did not significantly effect melatonin levels.

In conclusion, it appears that weight gain throughout adulthood impacts not only steroid receptor status, but also melatonin levels.

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# ASSOCIATION OF VARIATION IN DNA REPAIR GENES WITH BREAST CANCER RISK

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The XPD gene, a gene involved in nucleotide excision repair (bulky adducts), may be associated with altered protein function and altered DNA repair capacity. XRCC1 is involved in base excision repair. Alterations in the functions of these genes may be associated with an increased risk of breast cancer, especially among women exposed to ionizing radiation. To learn more about the association of breast cancer risk with these genes, we collected information about breast cancer risk factors, including extensive information about medical and occupational ionizing radiation exposure, and blood specimens for DNA extraction from 93 women with breast cancer, 120 women with a family history of breast or ovarian cancer (high-risk), and 131 comparison (control) women. We present results from SNPs (single nucleotide polymorphisms) in XPD exon 23, and XRCC1 exon 10.

Eighty-six percent of women with breast cancer were Caucasian, as were 90% of high-risk women and 59% of controls. Genotype frequencies for controls were in Hardy-Weinberg equilibrium for XPD and XRCC1, for both Caucasian and non-Caucasian women. Stratified analyses suggest different levels of association for Caucasian and non-Caucasian women. However, there were few non-Caucasian women with breast cancer (n=13) or at high risk (n=11). Preliminary results suggest that, among Caucasian women, XPD genotype is associated with breast cancer risk (Table 1), but XRCC1 may not be (Table 2).

Table 1. Association of variation in XPD with breast cancer risk

| XPD | Control | High-risk | OR | Breast cancer | OR |
|---|---|---|---|---|---|
| AA | 46.1% | 33.3% | 1.00 | 30.0% | 1.00 |
| AC | 43.4% | 46.7% | 1.48 | 55.0% | 1.94 |
| CC | 10.5% | 20.0% | 2.63 | 15.0% | 2.19 |

Table 2. Association of variation in XRCC1 with breast cancer risk

| XRCC1 | Control | High-risk | OR | Breast cancer | OR |
|---|---|---|---|---|---|
| CC | 49.2% | 51.1% | 1.00 | 39.7% | 1.00 |
| CT | 40.7% | 40.0% | 0.95 | 48.5% | 1.48 |
| TT | 10.2% | 8.9% | 0.84 | 11.8% | 1.43 |

We are currently analyzing the information on ionizing radiation exposure to assess the combined effect of genotype and exposure on breast cancer risk.

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# GENETIC DETERMINANTS OF AROMATASE EXPRESSION AND SUSCEPTIBILITY TO POSTMENOPAUSAL BREAST CANCER

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Estrogens play an important role in the etiology of postmenopausal breast cancer. Conversion of androstenedione to estrone in adipose tissue, catalyzed by the enzyme aromatase, is the major source of estrogen in postmenopausal women. We therefore hypothesized that factors regulating aromatase expression in adipose tissue may influence susceptibility to postmenopausal breast cancer. Aromatase expression in adipose tissue is regulated by the cytokines TNF-alpha and IL-6. We therefore proposed that polymorphisms in the IL-6 and TNF-alpha genes would influence (1) estrogen production, and (2) breast cancer risk in post-menopausal women.

Our objectives were to determine whether polymorphism in the TNF-alpha or IL-6 genes are associated with (1) the plasma estrone (E1) to androstenedione (A) ratio, a phenotypic measure of aromatase expression, or (2) risk of post-menopausal breast cancer. To test our hypothesis, we carried out a case-control study nested within the Hawaii-Los Angeles Multi-ethnic cohort study of diet and cancer. Women from four ethnic groups (Caucasian, African-American, Hispanic, and Japanese-American) were included in the study. Plasma hormone levels (E1 and A) were measured in post-menopausal control women (women with no history of breast cancer) who were not taking hormones. TNF-alpha and IL-6 genotypes were assayed on cases (women with post-menopausal breast cancer) and controls (women with no history of breast cancer).

To date, we have found that aromatase expression, as measured by the E1 to A ratio, is not influenced by TNF-alpha or IL-6 genotype. These results may indicate either that (1) polymorphism in the TNF-alpha and IL-6 genes does not influence aromatase expression, or that (2) the plasma E1 to A ratio is not a good indicator of aromatase expression in adipose tissue. Analyses to determine whether TNF-alpha and IL-6 genotypes influence risk of post-menopausal breast cancer are currently in progress. A finding that genetic differences influence breast cancer risk could lead to a better understanding of breast cancer etiology and could have important implications for breast cancer treatment and prevention.

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# RACE DIFFERENCES IN BREAST CANCER SURVIVAL

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Despite recent improvements in breast cancer mortality rates in the general population, race differences in breast cancer survival remain. Here, we investigate the prognostic significance of tumor characteristics, genetic alterations, medical care, psychosocial, and health /medical factors in survival in a cohort of African American (AA) and White (W) women diagnosed with breast cancer in the 1980s.

This is a population based follow-up study of 145 AA and 177 W women diagnosed between Jan, 1987 and May, 1989. As of Jan, 1999, 135 (41.9%) of the women had died. Women were followed for an average of 9.2 years. Survival among AA women (56.9%) was significantly lower than survival in W women (68.9%) [age-adjusted Risk Ratio {RR} 1.73 (95% Confidence Interval {CI} 1.21 – 2.48)].

AA women were twice as likely to be diagnosed with tumors that were TNM stage II or higher (age-adjusted Odds Ratio [OR] = 2.01, 95% Confidence Interval [CI] 1.24 – 3.24). Analysis of archived tumors showed numerous race differences, e.g., AA women were more likely than W women to have tumors that were higher histologic grade (age-adjusted OR 2.20, 95% CI1.08 – 4.49), higher nuclear grade (age-adjusted OR = 2.00, 95%CI 1.04 – 3.85), estrogen receptor negative OR = 1.82, 95% CI 1.09 – 3.03, and p53 positive (OR = 4.00, 95% CI 1.77 – 9.01). Results from Cox Proportional Hazard (CPH) multivariate models demonstrated that later stage at diagnosis was the key predictor of death due to all causes (Hazard Ratio [HR} 2.83, 95% CI 1.74- 4.6). Other factors that were associated ($p < .10$) with survival in models adjusted for tumor characteristics included older age, severe obesity at time of diagnosis, low income and some psychosocial factors (e.g., avoidance, no religious affiliation). In models adjusted for socioeconomic status, access to medical care, insurance coverage, comorbidity, severe obesity, smoking, treatment, and psychosocial factors, race / ethnicity no longer remained a significant predictor of all-cause mortality. This work demonstrates that race/ethnic disparities in breast cancer survival can be parsed out when considering the full range of possible predictors. Identification of key predictors represents an essential first step in lessening the cancer burden for AA women.

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# SNP IN PROHIBITIN 3'UTR AND BREAST CANCER SUSCEPTIBILITY

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The human prohibitin gene is located on chromosome 17q21 in a region near BRCA1 that frequently undergoes loss of heterozygosity (LOH) in both familial and sporadic breast cancers. The 3'untranslated region (3'UTR) of prohibitin codes for an RNA that arrests proliferation in normal mammary epithelial cells and certain breast cancer cell lines by blocking the G1 to S transition of the cell cycle. This RNA also has tumor suppressor activity in animal cancer models. We have discovered a 3'UTR single nucleotide polymorphism (SNP) that defines two human prohibitin alleles. The "C" allele codes for a regulatory RNA that inhibits cancer cell growth while the "T" allele product is inactive.

Our basic research led to the hypothesis that carriers (C/T or T/T) of the T allele have increased susceptibility to breast cancer. We did a case-control study of prohibitin genotype in 205 women recently-diagnosed with breast cancer and 1046 controls to determine if there was an association between prohibitin genotype and risk of breast cancer.

The current results showed an association between the T allele and breast cancer in women who reported a first-degree relative with the disease (Odds Ratio 2.5, $p = 0.005$). An even stronger association was found in a subset of women diagnosed at or before age 50 years (4.8, $p = 0.003$). These data suggest that prohibitin genotyping has value in assessing risk of breast cancer in women aged 50 years or younger with at least one first-degree relative with the disease. Because the T allele is common, prohibitin 3'UTR genotyping has the potential to become an effective screening tool for breast cancer risk.

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# GENETIC DETERMINANTS OF PLASMA IGF-I LEVELS

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Insulin-like growth factor-I (IGF-I) can enhance the development of tumors in different organs, including the breast. It is therefore important to understand what factors can lead to elevated IGF-I in the circulation and tissues. One well-documented determinant of circulating IGF-I levels is nutritional status, especially the availability of energy and essential amino acids from diet and body reserves. However, heritability studies comparing homozygous and heterozygous twins have shown that, in western populations, 40-60 % of variation in IGF-I is (co-)determined by genetic factors.

We have started a cross-sectional study to examine relationships of single-nucleotide polymorphisms (SNPs) in 14 candidate genes in the growth-hormone (GH) / IGF-I synthesis pathway. An exhaustive catalog of polymorphisms (coding and non-coding regions), was made by searching MEDLINE and genomic databases, and by experimental discovery using partially denaturing HPLC (DHPLC) in DNA samples from 137 Caucasian, 43 African, and 12 Japanese subjects. For 327 healthy men and women, aged 50 - 60, living in Umeå, Northern Sweden, these SNPs were typed using hybridization coupled with an enzyme-mediated primer extension on a DNA microarray. Of 60 SNPs typed successfully, 51 had a population prevalence above 1%, and no strong linkage disequilibrium with other SNPs ($\Delta<0.90$). For about 80% of individuals who were homozygous for all, or for all but one of the SNPs, SNP haplotypes could be calculated; for the remaining subjects haplotypes were estimated from population haplotype frequencies.

| Gene | Number of different alleles | | nr of SNP loci showing effects / model p-value / model $R^2$, for selected regression models |
|---|---|---|---|
| | SNPs | Haplotypes | |
| ***IGF1*** | 4 | 6 | 2 / p = 0.10 / $R^2$ = 0.03 |
| ***IGFBP3*** | 5 | 13 | 3 / p = 0.03 / $R^2$ = 0.04 |
| ***GH1*** | 3 | 4 | no significant effects |
| ***GHR*** | 5 | 7 | 1 / p = 0.06 / $R^2$ = 0.02 |
| ***GHRH*** | 2 | 3 | 1 / p = 0.06 / $R^2$ = 0.02 |
| ***GHRHR*** | 10 | 15 | |
| ***SST*** | 1 | | no significant effects |
| ***SSTR1*** | 1 | | no significant effects |
| ***SSTR3*** | 6 | 12 | 3 / p = 0.06 / $R^2$ = 0.05 |
| ***SSTR4*** | 4 | 9 | no significant effects |
| ***SSTR5*** | 4 | 9 | no significant effects |
| ***POU1F1*** | 1 | | no significant effects |
| ***GHRL*** | 4 | 9 | 2 / p = 0.08 / $R^2$ = 0.04 |
| ***GHSR*** | 1 | | 1 / p = 0.02 / $R^2$ = 0.02 |

Statistical models for phased SNP genotypes, selected by forward selection [$p_{IN} < 0.15$] and backward elimination [$p_{OUT} \geq 0.15$], showed evidence that polymorphic variation in the candidate genes may alter plasma IGF-I levels, although associations were only of borderline statistical significance, and the percent variation in IGF-I levels explained by individual gene variants was small. After increasing the sample size of the study, we plan to estimate a combined score for multiple candidate genes.

We expect this project to increase understanding of how cancer risk (breast and other organs) may be determined by dysregulations in the GH/IGF-I axis, and how such dysregulations may be codetermined by specific genetic susceptibility factors.

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# ESTROGEN METABOLISM-RELATED GENES AND BREAST CANCER RISK

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Endogenous estrogens are believed to play an important role in the etiology of breast cancer. Inherited variations in genes coding for metabolizing enzymes may result in inter-individual differences in the biosynthesis and degradation of estrogenic hormones and, thus, may affect breast cancer risk. Cytochrome P450 enzymes CYP1A1, CYP1A2, CYP1B1 and CYP3A4 metabolize estradiol to catechol estrogens, a group of compounds that are known to be mutagenic. The expression of the CYP1A1, CYP1A2 and CYP1B1 genes is regulated by the aryl hydroxylase receptor (Ahr). Catechol estrogens are inactivated by catechol-O-methyltransferase (COMT). These enzymes have been shown to be polymorphic.

The present study uses DNA samples collected as part of an on-going case-control study of post-menopausal breast cancer nested in a large cohort study of Caucasian, Japanese, Latino, African American and Native Hawaiian women residing in Hawaii and Los Angeles to test the following hypotheses: 1) The high activity alleles for CYP1A1, CYP1A2, CYP1B1, AHR, CYP3A4 and low activity allele for COMT are independently associated with increased breast cancer risk; 2) the risks associated with these polymorphisms are additive and carriers of multiple susceptibility alleles are at markedly elevated risk for breast cancer; and 3) associations with these genotypes are stronger in women whose past exposures to endogenous estrogens are predicted to be particularly high (e.g., those with early menarche, those who are overweight and/or those with more ovulatory years). The study uses PCR-based assays to genotype breast cancer cases and controls for the genes above.

To date, approximately1,400 cases and 1,400 controls have been genotyped. A preliminary analysis assessed breast cancer odds ratios adjusting for age, race and reproductive history. No main effect was detected for any of the polymorphisms studied. The analysis is now comparing the risks associated with these genes in combination, and among women with "high" and "low" exposure to endogenous estrogens, as predicted from risk factor information.

The identification of genetic susceptibilities may allow for the identification of women at high risk for breast cancer who could be the target for intense primary and secondary prevention.

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# INSULIN RESISTANCE, IGFS, AND ENERGY BALANCE ON THE RISK OF BREAST CANCER

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The purpose of this proposal is to research the association of insulin resistance and its joint effect with insulin like growth factors (IGFs) on breast cancer risk. Many epidemiological studies have investigated the association of body weight, fat distribution, and physical activity with the risk of breast cancer, and a few studies have compared levels of C-peptide (a measure of insulin resistance) and IGFs between breast cancer cases and controls. None of these studies, however, has evaluated the potential joint effect of C-peptide, IGFs and energy balance on the etiology of breast cancer.

Using data and biospecimens from a population-based case-control study of breast cancer, we propose to investigate the above hypotheses. The specific aims of this postdoctoral training proposal are 1) To determine blood levels of C-peptide and IGF1, IGF2, and IGFPB3 in a subset of subjects (400 case-control pairs) from the Shanghai Breast Cancer Study (R01CA64277) using pre-treatment blood samples, and to evaluate the association of blood C-peptide level and its joint effect with IGFs with the risk of breast cancer; 2) To analyze data collected from the Shanghai Breast Cancer Study to evaluate the association of energy balance with breast cancer risk; 3) To develop a proposal to investigate joint effects of C-peptide, IGFs, estrogens, and phytoestrogens on the risk of breast cancer. There is evidence to suggest that the combined effects of positive energy balance result in increased breast cancer risk and that C-peptide level is potentially related to the risk of breast cancer enhanced by IGF bioavailability.

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# GENETIC POLYMORPHISMS, ESTROGENS, AND BREAST DENSITY

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Mammographic density is a strong predictor of breast cancer risk and may be determined by circulating estrogens. Because some metabolites of endogenous estrogens have more estrogenic effects than others, genetically determined differences in biosynthesis and metabolic pathways of estrogens may affect breast cancer risk as reflected in mammographic densities. The purpose of this analysis was to investigate the relation of breast densities with the presence of polymorphic genes coding for hormone producing and metabolizing enzymes and the excretion of urinary estrogen metabolites in women of different ethnic background.

We have recruited 200 pre- and 100 postmenopausal healthy women who completed a dietary and medical history. The subjects donated a blood (90%) or a mouthwash (10%) sample and a urine specimen. So far, the following genetic polymorphisms have been analyzed for 93 women: CYP1A1 (MspI), CYP1A2 (intron 1), CYP1B1 (PstI), CYP17 (MspAI), and COMT (exon 4). We extracted DNA from whole blood and buccal cells with a rapid method using proteinase K digestion, phenol-chloroform extraction, and ethanol precipitation and analyzed the DNA samples by PCR/RFLP methods for the presence of the variant alleles. Competitive immunoassay kits are used to determine levels of 16α-hydroxyestrone and 2-hydroxyestrone in urine. After digitizing the mammographic films, the images were assessed for densities with a computer-assisted method. Percent density was calculated as the ratio of the dense areas and the total area of the breast.

The allele distribution for several polymorphic genes differed by ethnic background. Caucasian women were less likely to carry the variant allele for CYP1A1 than women with Asian and Hawaiian ancestry, whereas non-Caucasian women showed a higher proportion of common alleles for CYP1B1 and COMT than Caucasians. A preliminary inspection of the small sample available for analysis suggests some differences in mammographic density between women carrying the variant alleles as compared to women carrying the common alleles. Statistical significance of these results will be evaluated after data for all 300 samples is available. Identifying variant alleles may enable us to target prevention efforts toward women at high risk for breast cancer.

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# SEVERE CALORIC RESTRICTION DURING ADOLESCENCE AND RISK OF BREAST CANCER IN ADULT LIFE

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Conventional approaches in breast cancer prevention research have focused on women's adult life stages. The most susceptible period of mammary gland cells to external influences may be during puberty and adolescence when tissue levels of growth hormones reach lifetime heights and breast cells are dividing, but are not yet differentiated. Caloric restriction has an important protective role in experimental mammary carcinogenesis. Energy restriction might be crucial during adolescence when mammary tissue is especially susceptible to carcinogenic processes. This hypothesis is supported by the consistently observed association between height and risk of breast cancer. Anorexia nervosa is a severe illness that occurs generally during or just after puberty and is characterized by extremely low energy intake. We are studying the association between anorexia nervosa and the subsequent incidence of breast cancer in a cohort of Swedish women.

We are conducting a retrospective cohort study in Sweden. A cohort of exposed women, i.e., women who had been hospitalized for severe anorexia nervosa between 1965 and 1998 but survived the disease, has been retrospectively formed and is now followed for the occurrence of breast cancer by linkage with the Swedish Cancer Registry, the Swedish Death Registry, and the Emigration Registry using the National Registration Number, a unique identifier for each resident of Sweden. We ascertain cancers diagnosed after 1965, the date and cause of death after 1965, and date of censoring due to emigration. The observed cancer numbers are compared to the expected cancer incidence rates based on rates in the female Swedish population in 5-year age intervals during the same calendar time period.

We have identified 7303 women from the Swedish Inpatient Registry who were hospitalized for severe anorexia nervosa prior to age 40 between 1965 and 1998. We are currently in the process of linking these data with the Swedish Cancer Registry.

This research is important to the field of breast cancer research and to the public because it addresses the important questions whether (1) caloric restriction is important for mammary carcinogenesis in the human and (2) adolescence is a particularly susceptible period for the human breast.

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# GLUCOSE METABOLISM AND BREAST CANCER RISK: THE ORDET PROSPECTIVE STUDY

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There is some evidence that glucose and other factors related to glucose metabolism, such as insulin and insulin-like growth-factors (IGFs) may contribute to breast cancer development.

The present study analyzed the hypothesis that serum glucose, insulin levels, and insulin-like growth factor (IGF)-I pattern are associated with breast cancer using a nested case-control study. Between 1987 and 1992, 10,786 women aged 35-69 were recruited in a prospective study in Italy. Women with history of cancer and on hormone therapy were excluded at baseline. At recruitment, blood samples were collected after 12 hours fast between 7:30 and 9:00 AM from all study participants.

After 5.5 years, 144 breast cancer cases were identified among the participants of the cohort. Four matched controls were chosen for each breast cancer case from members of the cohort who did not develop breast cancer during the follow-up period.

In premenopausal women, glucose was associated with breast cancer risk: the age, BMI, and reproductive variable adjusted relative risk (RR) for the highest quartile of serum glucose versus the lowest was 2.8 [95% Confidence Interval (CI) 1.2 - 6.5], p for trend 0.02. Insulin showed a weaker association with breast cancer, the adjusted RR of the highest quartile versus the lowest was 1.7 (95% CI 0.7 - 4.1), p for trend 0.14, while the adjusted RR of the highest quartile of IGF-I was 3.1 (95% CI 1.1 - 8.6), p for trend 0.01. Increased levels of IGFBP-3 were related to breast cancer risk: the adjusted RR for the highest quartile 2.1 (95% CI 0.95 - 4.75), p for trend 0.02.

In postmenopausal women, none of the variables was associated with breast cancer risk. These results indicate that chronic alteration of glucose metabolism are related to breast cancer development in premenopausal women.

Acknowledgment: We thank all women participants in the ORDET prospective study. Their participation in the study has allowed a more clear understanding of several potential etiological pathways of breast cancer development.

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# ASSOCIATION OF TWO HPC2/ELAC2 MISSENSE VARIANTS WITH RISK OF BREAST CANCER

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BACKGROUND. The first prostate cancer susceptibility gene ELAC2 (HPC2) was cloned in 2000, and two common missense variants of the gene, Ser217Leu and Ala541Thr were found to be associated with both familial and sporadic forms of the disease. Both breast and prostate cancers are hormonally regulated, and elevated risks of prostate cancer are seen in families with multiple cases of breast cancer. Our objective was to determine whether variants in HPC2/ELAC2 gene were associated with breast cancer risk or tumor stage and grade at diagnosis.

METHODS. We examined 375 familial breast cancer cases diagnosed at less than 71 years of age. These cases consisted of 121 BRCA1 and 35 BRCA2 mutation carriers, and 219 breast cancer cases without BRCA1 and BRCA2 mutations. Controls were women without cancer, including BRCA1/2 carriers and non-carriers. The cases carrying BRCA1 and BRCA2 mutations were analyzed together with the 219 cases without mutations and separately as a nested case-control within a cohort of mutation carriers. We used allele specific PCR to genotype DNA for the Ser217Leu and Ala541Thr variants.

RESULTS. The HPC2/ELAC2 variants were not associated with risk of breast cancer. However, women homozygous for the Leu217 variant were diagnosed with breast cancer earlier than those with one or no copies of Leu217 (t-test, 4.05 years, p=0.036 and 4.76 years, p=0.013, respectively). Kaplan-Meier survival analysis also indicated a significantly earlier age at diagnosis in cases carrying more Leu217 alleles (p = 0.011). Thr541 genotypes were not associated with earlier age at diagnosis. However, a significant excess of Thr541 carriers in cases with advanced clinical stages was observed (p=0.021).

CONCLUSION. These results suggest that the HPC2/ELAC2 missense variants may be associated with earlier age at diagnosis and more advanced stage of breast cancer in women from high-risk breast cancer families, regardless of whether they carry BRCA1 or BRCA2 mutations. Other studies need to be performed to confirm these results.

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# 4-AMINOBIPHENYL-DNA ADDUCT DAMAGE IN BREAST TISSUE AND THE RELATIONSHIP TO POLYMORPHISMS OF METABOLIZING GENES

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Only a few epidemiological studies have shown a slight increased risk of breast cancer in cigarette smokers. The risk is only seen when variables of smoking status or genotypic variance in metabolizing enzymes are considered. To evaluate DNA damage due to smoking exposure we studied 4-aminobiphenyl(4-ABP)-DNA adduct levels in breast tumor tissue in a population-based study of 150 cases from New Jersey. All subjects were under the age of 45 with either *in situ* or invasive breast cancer. Adducts in breast tumor sections of paraffin blocks were measured using an immunohistochemical peroxidase assay. The difference in relative staining intensity for 4-ABP-DNA adducts in tumor tissue was not significantly different between smokers 0.31 (0.17), ex-smokers 0.28 (0.16), and non-smokers 0.29 (0.15). 4-ABP-DNA adduct levels were not significantly elevated in smokers with higher compared to lower exposure in relation to age at initiation or pack-years. This data suggests that exposure to cigarette smoke might have an affect on breast cancer risk.

4-ABP is not carcinogenic in the parent form but requires metabolic activation to reactive electrophiles in order to produce its carcinogenic effects. We evaluated single nucleotide polymorphisms in the enzymes that have been found to metabolize 4-ABP; cytochrome P4501A2 (CYP1A2), two N-acetyltransferases, NAT1 and NAT2, and glutathione-S-transferase(GST).

None of these genes, independently or in combination, had a significant association with adduct levels. Passive smoking exposure data was not available in the parent study possibly causing a misclassification of nonsmokers and may have contributed to the lack of associations. This data also suggests that breast tissue may not be an appropriate medium to assess breast cancer risk with DNA damage levels associated to cigarette smoking exposure. Finally, this particular population is young and the inheritance of mutations in high-risk genes, such as BRCA1 and BRCA2, may play a larger role in breast cancer development rather than environmental exposures.

The conclusions drawn from this project have shown that cigarette smoking may have an affect on breast cancer risk and DNA damage levels in breast tissue is not modified by individual susceptibility markers of carcinogen metabolism.

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# BREAST CANCER RISK ASSESSMENT IN CLINICAL POPULATIONS: DEMONSTRATION OF A COMPUTERIZED METHOD OF PRELIMINARY RISK SCREENING

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Preliminary stratification of women according to breast cancer risk level is now possible using several statistical risk models. We have developed a computerized risk assessment program, BRISK, that calculates interval breast cancer risks using the Gail and Claus epidemiological models, and BRCA1 and BRCA2 mutation probabilities using the Couch, Shattuck-Eidens, Frank, and BRCAPRO models. The BRISK program was used to perform brief breast cancer risk assessments (BRA) in the diagnostic and treatment clinics of the Comprehensive Breast Program of the University of Pittsburgh Cancer Institute/Magee-Womens Hospital.

We utilized BRA as a method of risk-based screening for referral to a cancer genetic counseling service. Questionnaires assessing psychological status, and knowledge and attitudes about breast cancer, cancer risk counseling, and genetic testing were used to identify predictors of referral uptake. Of the 120 subjects in the biopsy setting, 53% had breast cancer risk $\geq$ twice the population risk as measured by the Gail and Claus models. Of the 91 women in the treatment setting, 47% had a BRCA mutation risk $\geq$ 10%. Uptake of referral to cancer risk counseling was low in the biopsy group (1/63), but higher in the treatment group (13/43). Predictors of uptake included family history of cancer, interest in family members' risks, high income, psychological disturbance, and perceived risk. Barriers included lack of time, cost, and fear of insurance discrimination. However, 81% thought BRA should be routine, suggesting annual mammography or OB/GYN visits as the most effective setting.

Studies to date have focused on uptake of genetic counseling and testing offered to women as part of cost-free research studies. Uptake of referral may be less in the clinical arena and may depend on timing or setting of the referral. Brief breast cancer risk assessment is easily incorporated into clinical settings and is well-accepted by most patients. It can be utilized not only for management of individuals, but also for public health intervention, resource allocation, and targeted research.

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# ANALYSIS OF TWIN DATA ON MULTIPLE CANCERS USING REPEATED MEASURE METHODS

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A new method for the simultaneous genetic analysis of two or more discrete traits such as the presence of breast and ovarian cancers in twins will be presented. A generalized estimating equations (GEE) logistic regression model will be used for the modeling. The methodology is motivated from the Hardy-Weinberg Law and uses the twin odds ratio as a measure of within-pair similarity for a dichotomous trait. A shared trait is defined for two discrete traits based upon explicit patterns of trait concordance and discordance within twin pairs; this shared trait is assessed for the influence of additive genetic and/or common environmental effects. Data are summarized in the form of 2 x 2 tables (for monozygotic and dizygotic twins) by combinining appropriate cells from the 16-cell multinomial distribution to define the individual and shared trait. Hypothesis tests for additive genetic and common environmental influence are performed using repeated measures logistic regression via the GEE approach. The model specification is highly flexible, accounts for the correlated structure of the parameter estimates and does not require multivariate normality assumption for the underlying liability distribution. The approach is illustrated using two example data sets from the Vietnam Era Twin (VET) Registry.

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# GENETIC PROFILE OF BREAST CANCERS IN WOMEN EXPOSED IN UTERO TO DIETHYLSTILBESTROL (DES)

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Estrogen is suspected to play a role in breast tumorigenesis. The timing of potentially damaging exposures is uncertain. Prenatal influences may be of importance, since before birth the mammary gland exists in a partially undifferentiated state and might be susceptible to intrauterine influences increasing the risk of cancer in the adult. Diethylstilbestrol (DES), a synthetic estrogen prescribed to pregnant women in the 1940s –1960s, is a transplacental carcinogen, leading to reproductive tract malignancies in women exposed prenatally. Cancer incidence in other organs is under investigation. DES' effects may be due to genotoxicity, such as aneuploidy and microsatellite instability (MI). To test the hypothesis that in utero DES exposure leads to characteristic genetic abnormalities in breast cancers, we examined archived breast cancers from consenting subjects enrolled in the NCI's Continuation of Follow-Up of DES Exposed Cohorts Study. This study follows exposed women and matched controls. We retrieved blocks, cut sections, microdissected multiple areas of cancer and normal epithelium and extracted DNA. 28 cases (21 exposed, 7 unexposed) were evaluable; each sample from these 28 cases was examined with 20 selected microsatellites.

We found that the incidence of MI in these samples was negligible (1 instance of a novel allele). This differs from findings in cervico-vaginal clear cell carcinomas associated with DES exposure, but is consistent with reports that MI is uncommon in breast carcinomas. In contrast to the absence of MI, LOH was seen in all cancers, with no overall effect of exposure on degree of LOH. (Proportional LOH in exposed vs unexposed CIS= 0.38 vs 0.32 ($p = 0.7$); and in invasive cancers = 0.42 vs 0.36 ($p = 0.4$)). Comparing LOH at specific arms suggested trends towards more LOH at 17p ($p = 0.08$) and 11p ($p = 0.09$), and less LOH at 16q ($p = 0.1$) in exposed vs unexposed cancers.

Thus, important genetic features of breast cancer appear similar in exposed vs unexposed. DES' effects may therefore be tissue-specific, which is reassuring to exposed women. However, it remains possible that pathways (involving ts genes at 17p, 11p and/or 16q) by which aberrant estrogen exposure contributes to breast carcinogeneis may exist and be identified.

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# SOY FOOD INTAKE, INSULIN-LIKE GROWTH FACTOR-I, AND BREAST CANCER RISK

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Previous reports from the Shanghai Breast Cancer Study (SBCS) suggested that adolescent and adult soyfood intake was inversely related to the risk of breast cancer, and elevated levels of insulin-like growth factor I (IGF-I) were associated with an increased risk of breast cancer. In the current study, we assessed whether IGF-I levels modified the effect of soyfood intake on breast cancer risk.

The SBCS is a population-based case-control study of breast cancer among women age 25 to 64 conducted between 1996 and 1998 in urban Shanghai. In-person interviews were completed with 1459 incident breast cancer cases ascertained through a population-based cancer registry, and 1556 controls randomly selected from the general population in Shanghai (with respective response rates of 91% and 90%). This analysis is restricted to the 300 cases and 300 matched controls for whom information on IGF-I levels was available.

After adjustment for confounding, the protective effect of soyfood intake was only observed among women with a lower IGF-I level (OR= 0.56 for adolescent and OR=0.68 for adult soyfood intake) but not for women with a higher IGF-I level (OR=1.41 for adolescent and OR=2.12 for adult soyfood intake), although none of the point estimates was statistically significant, possibly due to the small sample size. Higher IGF-I level was associated with an increased risk of breast cancer regardless of the level of soyfood intake.

Our results appear to suggest that the effect of soyfood intake on breast cancer risk is dependent on adult IGF-I levels. Further studies are needed to confirm our finding and to understand the biological mechanism of this possible interaction. Should IGF-I levels prove to be associated with breast cancer in other populations, the possibility that soyfood intake may modify this association will be useful in targeting interventions for women at high risk for breast cancer.

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# P53 GENE MUTAGENESIS IN BREAST CANCER

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The incidence and mortality from breast cancer varies significantly in ethnically and geographically distinct populations. Patterns of p53 gene mutations in breast cancers are different among several ethnically and/or geographically distinct populations. We hypothesize that variability in the patterns of p53 mutagenesis in breast cancer reflects differences in exposures to different amounts and/or types of diverse environmental mutagens. We postulate that mammary cells are sensitive to a diversity of lipophilic mutagens in the diet because of the unique architecture of breast tissue, i.e., tiny islands of cancer-prone rapidly-dividing mammary cells surrounded by a sea of fat cells. It has now become possible to measure mutation load in an individual by identifying p53 mutations in the normal mammary cells. The normal breast tissue is obtained away from the tumor margin in women with breast cancer. With this newly developed technique, it is possible to determine the mutation fingerprint in each woman and correlate this with her diet. Single cells stained immunohistochemically for p53 protein overabundance were microdissected from paraffin-embedded tissues and examined for rare heterozygous mutations in the p53 gene. A 1.8 kb segment of the p53 gene (exons 5 to 9) was amplified with an 87% success rate from single cells. About half of the amplified stained cells had missense mutations (51%). Allele dropout, a serious concern in amplification from single cells, occurred in 40% of amplified cells containing a known polymorphism. False positive sequence changes were observed in 17% of the unstained cells presumably due to DNA polymerase error. The ability to measure individual mutation load greatly increases the power to test the central hypothesis. We propose to test twenty upper Midwest U.S. women with breast cancer, ten with diets high in animal fat and ten with diets low in animal fat. In each woman, 30 different mutations will be defined in mammary cells. It is hypothesized that there will be substantial individual variation in mutation patterns within the population and that mutation load will correlate with the amount and type of fat consumed in the diet consistent with key predictions of the “lipophilic mutagens hypothesis”.

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# LIPOPHILIC MUTAGENS, FAT CONSUMPTION, AND BREAST CANCER

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The origins of sporadic breast cancer are largely unknown. Lipophilic mutagens in the diet may play a role in the etiology of breast cancer. The rationale for this is based upon four observations. 1) The pattern of p53 mutations in human breast cancer differs in the majority of 15 populations including cancer-prone and cancer-resistant populations. 2) Descendants of migrants from countries with a low-incidence of sporadic breast cancer have incidence rates of breast cancer close to those of the new country. 3) The anatomy of breast tissue may predispose mammary epithelium to mutations from lipophilic mutagens concentrated in adjacent adipocytes. 4) Recent in vitro assays for mutagenicity showed 40% of lipid extracts from human breast tissues were mutagenic. Four specific aims test the central hypothesis that mammary epithelium is differentially susceptible to lipophilic mutagens preferentially concentrated in adjacent adipocytes and originating in the diet. 1) Demonstrate if lipophilic mutagens delivered orally preferentially accumulate in adipocytes and specifically cause increased mutation frequencies and altered mutation patterns in mammary epithelium. 2) Determine if normal and malignant human mammary tissue from cancer-prone populations and animal fat are mutagenic in an in vitro mutagenicity assay. 3) Determine the in vivo mutagenicity of lipid extracts from normal and malignant human mammary tissues and animal fat. 4) Demonstrate that mouse diets high in animal fat contain diverse lipophilic mutagens that upon chronic exposure are concentrated in mouse adipocytes and cause mutations in mammary epithelium. The Big Blue mouse mutation detection system permits direct assay of mouse-derived mutations in the lacI reporter transgene. The frequency and pattern of mutation will be examined in mammary, adipose and skin tissues. The proposed experiments will analyze the mutagenicity of lipid extracts from primary human tissues as well as dietary animal fats that are likely sources of lipophilic mutagens. The results have implications for elucidating the etiology of breast cancer and providing passive (avoidance of fat) or active (reduction in mutagenic exposure by altering the diet that farmers feed livestock) avenues for its prevention.

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# LEPTIN (OBESITY PROTEIN) IN NIPPLE ASPIRATES AND BREAST CANCER RISK

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The link between obesity and breast cancer development has been postulated but the molecular mechanisms involved are unknown. Leptin, the product of the ob (obesity) gene is a cytokine controlling food intake and energy balance by sensing nutritional state and providing information to the central nervous system. In addition to this principal function, leptin has been shown to act as an angiogenic factor and play a role in neovascularization of tissues. Leptin has also been implicated in migration and invasion of epithelial cells. Although leptin is secreted mainly by adipocytes, recent data documented that breast cancer cells can express leptin mRNA and protein.

Leptin synthesis is greater in females than in males, and is regulated by endocrine factors, such as estradiol and insulin. The levels of these hormones are elevated in individuals with upper body obesity. This type of obesity correlates with increased breast cancer risk in post-menopausal women. We hypothesized that in obese women, locally elevated levels of estrogens and insulin could increase the synthesis of leptin by mammary epithelial cells (normal or malignant). In consequence, high levels of leptin could promote metastatic processes (neovascularization and invasion).

We began studies to assess whether leptin produced locally in the breast correlates with obesity and breast cancer risk. Leptin was measured in nipple aspirate fluid (NAF) obtained by a non-invasive procedure from breast cancer patients and healthy women. The initial study population consisted of 33 individuals with body max index (BMI) ranging from 19.3 to 40.8 and breast cancer risk ranging from no risk to invasive breast cancer. Leptin concentration in NAF samples, as measured by non-radioactive human leptin ELISA kit (Linco Research), ranged from non-measurable to ~50 ng/ml.

In the preliminary experiments, a trend towards high levels of NAF leptin in obese individuals was observed. The success (%) of measuring leptin in NAF in relation to BMI was as follows: BMI<20, 0%; BMI 20-24, 35%; BMI 25-29.9, 29%; BMI >30, 100%. In summary, leptin can be measured in a significant subset of NAF samples, with levels increasing with higher BMI. Our future studies will address the association of NAF leptin with breast cancer risk.

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# METABOLIZING ENZYME POLYMORPHISMS AND PROGNOSIS AMONG WOMEN TREATED FOR BREAST CANCER

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Background: Despite the favorable prognosis for women treated for breast cancer, in some instances cancer will recur, presumably because some tumor cells survive the primary therapy. Therapy for breast cancer often includes cyclophosphamide, adriamycin, and radiation. Variability in enzyme activities could be a factor influencing sensitivity of cells to these treatments. Common polymorphisms occur in several genes encoding drug metabolizing enzymes, and the variant alleles affect enzyme activities. Glutathione S-transferase (GST) A1 and GSTP1 enzymes catalyze inactivating glutathionyl conjugation reactions of cyclophosphamide intermediates. The GSTA1*B variant reduces expression of GSTA1, while a GSTP1 Val105 variant reduces specific activity toward alkylating agents, so these polymorphisms may improve treatment effect by reducing removal of the drug. Data from our pilot study indicate that among women treated for breast cancer, those who are homozygous for GSTP1 Val105 or GSTA1*B have improved overall survival. In a cohort of women treated for breast cancer, we will investigate whether presence of inherited variant alleles affecting activity of metabolizing enzymes affect overall survival or time to recurrence outcomes.

Study Design: Women (n=700) receiving first course of therapy for invasive, primary breast cancer at one hospital will be identified, and information on vital status and recurrence will be obtained from registry follow-up data. We will determine genotypes using DNA extracted from normal tissue from archived surgical blocks. We will assess other prognostic markers in tumor tissue by immunohistochemistry. We will use survival analysis methods, taking into account other prognostic factors, to evaluate associations between genotypes and recurrence and overall survival.

Relevance: There are now several categories of adjuvant therapy with proven benefit for breast cancer patients, including radiation, polychemotherapy, hormonal therapy, and taxanes. If subgroups of patients, because of inherited genotypes for metabolizing enzymes, have the most potential to benefit from a particular type of therapy, then in future the patient's pattern of inherited enzyme variants may be considered in deciding on the best course of therapy.

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# ALTERED EXPRESSION OF MHC CLASS I AND II IN BREAST CANCER

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It has been known for some time that malignant transformation of cells is frequently associated with abnormalities in the expression of MHC class I antigens. These abnormalities appear to play a role in the clinical course of the disease and to have a negative effect on the outcome of T cell-based immunotherapy for malignant diseases. Down regulation of HLA class I antigens in breast carcinomas may be more frequent than previously reported suggesting that alterations of HLA class I could represent an important step associated with tumor invasion providing tumor cells with the ability to escape recognition by T-lymphocytes.

As an initial step, a multicenter study was undertaken to standardize our class I and II protocols using formalin-fixed paraffin-embedded tissues (FFPTs). Class I and II expression was concordantly reported by four participating laboratories with the anti-HLA class I antibody HC10, anti-B2 microglobulin L368, and anti-HLA class II LGII. The results indicate that FFPTs represent a useful substrate upon which to monitor HLA antigen expression in malignant lesions, especially when appropriate markers are used to differentiate malignant cells from lymphocytes and dendritic cells.

An initial cohort of 44 breast cancer cases showed an altered expression of class I HC10 in 40/44 cases: 27 showed down regulation (decreased expression or complete loss), 8 upregulation (overexpression), 5 heterogeneity for loss, and 4 indicated no change from normal expression. Loss of B2-microglobulin expression (L368) was observed in 18/33 cases. Concordance for altered expression of HC10 and L368 was noted in 23 cases, the most frequent being loss (14/23).

A relationship was found between HLA class I antigen expression and the degree of differentiation of malignant cells. Generally, neither breast carcinoma cells nor normal mammary epithelial cells were stained by anti-HLA class II mAb LGII. Lymphocytes and dendritic cells were the consistently stained by mAb LGII in the tissue sections analyzed, however, heterogeneous or upregulation was observed in 6/33 cases stained for LGII.

These findings suggest that abnormalities in HLA class I and Class II antigen expression are frequently associated with malignant transformation of mammary epithelial cells.

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# MOLECULAR EPIDEMIOLOGY OF BREAST CANCER: DEVELOPMENT AND VALIDATION OF ACETYLATION METHODS FOR CARCINOGEN-DNA ADDUCT DETECTION

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Molecular epidemiology can elucidate new breast cancer risk factors and gene-environment interactions relating to both hormonal and non-hormonal carcinogenic mechanisms. Corroborative epidemiological studies of intermediate biomarkers of carcinogenesis and laboratory studies demonstrating functional importance of the epidemiology findings are needed. The study of carcinogen-DNA adducts can provide corroborative evidence for the importance of genetic susceptibilities in breast cancer risk. We are establishing new assays for the detection of carcinogen-DNA adducts, use it for the first time in humans, and rigorously validate it to prove its utility for human breast tissue analysis in epidemiological studies, and determine adduct levels in relation to metabolizing gene polymorphisms. Two assays are under development, one for benzo(a)pyrene (BP) and the other for 4-aminobiphenyl (4ABP). The first is based on capillary HPLC and laser-induced fluorescence. This method, due to the substantially improved technology, is more sensitive and easier to do compared to earlier methods. We have been able to detect the adducts in human samples and are now validating the method. The 4ABP assay is highly novel because it is an enzymatic radiolabeling method that uses commercially available N-acetyl-transferase, so that we have high specificity. The adducts are labeled with 14C acetyl CoA and then the level of radioactivity is quantitated by accelerator mass spectroscopy (an ultrasensitive 14C detection unit). Because these DNA adducts are the result of metabolic processes, we have been determining cytochrome P450 levels in human breast tissue, thus far detecting CYP1B1, and others are in progress. The third major aspect of this project is to develop primary human breast cell cultures and determine the metabolic capacity and cellular responses to DNA damage. We have seen wide variability in p53 response among women. With all these, we will apply the methods to surgical breast tissues and relate these different parameters as they relates to age, gender, race, and smoking. We have breast tissues from 235 donors (200 women, 35 men), and have established cell strains from 40 of them. In the end, we will have determined several related complex phenotypes from women, in an epidemiological context. These studies can then lead to the study of the genetic bases for these phenotypes. Thus, this study is important because it holds the promise of identifying several new genetic traits for breast cancer risk.

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